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Study Title

Toxicity of Soil from the Somers, MT Landfarm to
Photobacterium phosphoreum, *Lactuca sativa*, and *Eisenia foetida*

Author

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Studies Completed On

July 13, 1993

Performing Laboratory

ENSR Consulting and Engineering
Fort Collins Environmental Toxicology Laboratory
1716 Heath Parkway
Fort Collins, CO 80524

Laboratory Project ID

8505-093-124





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October 27, 1993

Lena Blais
Remediation Technology, Inc.
1011 S.W. Klickitat Way
Suite 207
Seattle, WA 98134

Dear Lena:

Mark Stromberg requested that I send you a copy of the report on the July toxicity tests performed on soils you collected in June at the Somers, MT landfarm. Individual soil samples were analyzed using Microtox; a composite of all samples was evaluated using lettuce seed germination and earthworm survival.

Please give me a call if you have any questions about these tests.

Sincerely,

A handwritten signature in black ink, appearing to read "David A. Pillard".

David A. Pillard, Ph.D.
Aquatic Ecologist/Toxicologist

DAP/lel

enclosure

File: 1140-020

ENVIRONMENTAL
PROTECTION AGENCY

NOV 17 1993

MONTANA OFFICE

STATEMENT OF QUALITY ASSURANCE

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol or other appropriate guidelines and standard operating procedures. This report is an accurate reflection of the raw data.

Anita Ene
Quality Assurance Unit

August 31, 1993
Date

SUMMARY

Sponsor	Burlington Northern Railroad 9401 Indian Creek Parkway Overland Park, KS 66201
Project Officer	Mark Stromberg (913) 661-7016
Study Director	David A. Pillard, Ph.D. (303) 493-8878
Senior Biomonitoring Technician	Stan W. Capps
Test Facility	ENSR Consulting and Engineering 1716 Heath Parkway Fort Collins, Colorado 80524
Location of Data	Data Records and Storage 328 Link Lane #4 Fort Collins, Colorado 80524
Test Substance	Soil
Test Endpoint(s)	Microtox: EC ₅₀ <i>Lactuca sativa</i> Seed Germination: EC ₅₀ and NOAEC <i>Eisenia foetida</i> Toxicity Test: LC ₅₀ and NOAEC
Test Duration	15 Minutes (Microtox) 5 Days (Seed Germination) 14 Days (Earthworm Survival)
Test Dates	June 16 and 17, 1993 (Microtox) June 16 to June 21, 1993 (Seed Germination) June 29 to July 13, 1993 (Earthworm Survival)
Test Species	<i>Photobacterium phosphoreum</i> (luminescent bacteria) <i>Lactuca sativa</i> (Lettuce, Buttercrunch) <i>Eisenia foetida</i> (Earthworms)
Source of Organisms	Microbics Corporation (<i>Photobacterium phosphoreum</i>) Rocky Mountain Seed Company (<i>Lactuca sativa</i>) ENSR In-House Culture (<i>Eisenia foetida</i>)
Test Concentrations	0 (Control), 0.4, 2.0, 10.0, and 50.0% Soil Extract (Microtox Assays) 0 (Control), 6.25, 12.5, 25, 50 and 100% of the Test Material (Seed Germination and Earthworm Survival)

1.0 INTRODUCTION

Toxicity tests were conducted at ENSR Consulting and Engineering's Fort Collins Environmental Toxicology Laboratory (FCETL) to determine the toxicity of test soils collected at the Burlington Northern Somers, Montana Landfarm, to marine luminescent bacteria (*Photobacterium phosphoreum*) (Microtox), buttercrunch lettuce (*Lactuca sativa*), and earthworms (*Eisenia foetida*). The criteria for effects were decreased bacterial luminescence of *Photobacterium phosphoreum* in the Microtox tests, reduction in germination and/or root length in the lettuce test, and survival in the earthworm test. Microtox (*Photobacterium phosphoreum*) test results are expressed as the median effect concentration (EC_{50}), which is the percent of sample which produces a 50 percent reduction in luminescence at the specified time of exposure. Lettuce test results also are expressed as the EC_{50} (the concentration of material estimated to produce a 50 percent reduction in germination or root elongation in the specified time of exposure) and the NOAEC (No Observable Adverse Effect Concentration). Earthworm test results are expressed as percent survival in the specified time of exposure. The median lethal concentration (LC_{50}), which is the calculated concentration of material which causes 50 percent mortality in the earthworm population at the specified time of exposure, and the NOAEC were also determined.

2.0 MATERIALS AND METHODS

2.1 Test Material

The test material was delivered to the FCETL via Federal Express on June 7, 1993. Five samples consisting of two 1-liter glass jars each, labeled LTF-A, LTF-B, LTF-C, LTF-D, and LTF-E, were designated FCETL sample #4842, #4843, #4844, #4845, and #4846, respectively. The samples were mixed well to assure homogeneity, and tested individually (i.e., five separate tests) using the Microtox methodology. Subsequently, all five samples were composited and homogenized, creating a single test material, for the lettuce and earthworm tests. A chain of custody form accompanied the samples and is presented as Appendix A.

2.2 Microtox Test

2.2.1 Control Water

The control water was Microtox diluent (a 2 percent NaCl solution in purified water) manufactured by Microbics Corporation.

2.2.2 Soil Extract

The soil extract was prepared by placing 25 g of the soil sample in a mason jar with 100 ml Milli-Q water, sealing the jar with a foil-lined cap, and tumbling the mixture for 18 to 22 hours at 40 rpm. The soil/water mixture was then allowed to settle for approximately 30 minutes, after which 15 mls of the extract was drawn off and centrifuged. The salinity of 10 mls of the supernatant was adjusted by adding 0.2 g NaCl. A duplicate sample was prepared and analyzed concurrently.

2.2.3 Test Organisms

The Microtox reagent is a lyophilized strain of marine luminescent bacteria most closely resembling *Photobacterium phosphoreum* in its characteristics. The reagent used in testing was Microbics Corp. Lot #AM018 - expiration 5/94. Prior to use in the test, the reagent was stored in the freezer compartment of a laboratory refrigerator. The reagent was prepared for use in testing by adding 1 ml Microtox Reconstitution Solution to the vial containing the lyophilized reagent. The reconstituted reagent was transferred to a Microtox cuvette and mixed by aspirating and dispensing 20 times with a 500 μ L micropipettor. The reagent was stored in the pre-cool well of the Microtox Analyzer (Model 2055) throughout testing.

2.2.4 Test Methods

The tests were conducted according to Beckman Instruments (1982) guidelines. A quality assurance standard test (using 10 mg/L sodium pentachlorophenol (SPP)) was run with each vial of reagent used in testing. Test chambers were Microtox standard disposable cuvettes manufactured from borosilicate glass. The test concentrations were 6.25, 12.5, 25, and 50 percent for the SPP standard tests, and 0.4, 2.0, 10.0, and 50.0 percent for the soil extract tests with two replicates per treatment group for each of the tests. A control treatment was conducted with each test. The test chambers were held in the incubator wells of the Microtox Analyzer at 15°C throughout testing.

The test chambers were filled with 500 μ L Microtox diluent, and 10 μ L Microtox reagent was added to each test chamber. After a ten minute equilibration period, the test chambers were cycled through the turret assembly of the Microtox Analyzer (which contains a photomultiplier tube) to obtain initial (T_0) light level readings. The test material was then added to the test chambers by transferring 500 μ L of pre-mixed solutions of the soil extract and the control water to the test chambers. Five minutes later, the test chambers were cycled through the turret assembly to obtain T_5 light level readings. Light level readings were obtained once again at time T_{15} (ten minutes after the last readings).

2.2.5 Data Analysis

The EC_{50} values, r^2 values, and confidence intervals were calculated using a Lotus 1-2-3 spreadsheet.

2.3 Buttercrunch Lettuce (*Lactuca sativa*) Seed Germination Test

2.3.1 Control/Hydration Water

The control/hydration water was dechlorinated city water that was passed through the laboratory's Milli-Q system.

2.3.2 Artificial Soil

The artificial soil used in the study was commercially available, washed silica sand (16-40 mesh). Once seeds were placed on the surface of the sand, and it was hydrated, a layer of silica sand of a larger mesh size (10-20) was placed over the seeds.

2.3.3 Test Organisms

Lactuca sativa seeds were obtained from Rocky Mountain Seed Company, lot #7578. Prior to testing, the seeds were size-graded and passed through a seed blower at the National Seed Storage Laboratory at Colorado State University to remove debris and empty seed hulls. Seeds were stored at 4°C prior to test initiation.

2.3.4 Test Methods

The test was conducted in general accordance with USEPA (1989) guidelines, with some modifications. Those modifications include use of smaller testing chambers, fewer seeds, and a lower volume of hydration liquid. Testing was conducted in 100 x 15 ml Petri dishes containing 50 g of test material appropriately diluted with artificial soil. Each test concentration was

hydrated to 75 percent of its calculated water holding capacity with Milli-Q water. A control, also hydrated with Milli-Q water, was conducted concurrently. At test initiation, ten seeds were randomly distributed to each test chamber and three replicates were tested per treatment concentration. The larger-grain sand was placed over the seeds. The test chambers were placed on trays inside black, plastic bags. The bags were sealed with tape and the trays were placed in an environmental chamber. After 48 hours the trays were transferred to clear bags for the remainder of the test. The test was conducted at 25°C under fluorescent lighting with a photoperiod of 16 hours light and 8 hours dark.

2.3.5 Data Analysis

Percent germination and mean root lengths were determined. The EC_{50} values were determined by the Trimmed Spearman-Kärber method. The NOAECs (No Observable Adverse Effect Concentrations) for germination and for root length were determined using analysis of variance followed by Dunnett's test. Normality and homogeneity of variance were first confirmed using Shapiro-Wilk's and Bartlett's test, respectively.

2.4 Earthworm Survival Test

2.4.1 Control/Hydration Water

The control/hydration water was dechlorinated city water that was passed through the laboratory's Milli-Q system.

2.4.2 Artificial Soil

The artificial soil used in the study consisted of (by weight): 10 percent - 2.36 mm screened sphagnum peat, 20 percent - colloidal kaolinite clay, and 70 percent - silica sand (40-140 mesh). Prior to use in the test, the artificial soil was well mixed, and the pH was adjusted using $CaCO_3$.

2.4.3 Test Organisms

Earthworms (*Eisenia foetida*) were obtained from in-house cultures. Prior to testing, the earthworms were examined to assure all were adults (i.e., possessed a clitellum); and a subsample of ten earthworms was weighed to determine pretesting mean weights.

2.4.4 Test Methods

The test was conducted in general accordance with USEPA (1989) guidelines, with some modifications. Those modifications include fewer organisms per replicate and a greater number of replicates. Testing was conducted in glass mason jars (of sufficient size to provide a loading rate of not less than 40 to 50 g soil per 1 g earthworm tissue) containing 200 g of test material appropriately diluted with artificial soil. Each test concentration was hydrated to 75 percent of its calculated water holding capacity with Milli-Q water. A control, also hydrated with Milli-Q water, was conducted concurrently. At test initiation, five earthworms were randomly distributed to each test chamber and three replicates were tested per treatment concentration. The test chambers were covered with tight-fitting lids with air holes and placed in an environmental chamber. The test was conducted at $22 \pm 3^{\circ}\text{C}$ under continuous fluorescent lighting.

2.4.5 Data Analysis

Percent survival and mean weights of surviving organisms were determined. The 14-day LC_{50} value was calculated using the probit method. Shapiro-Wilk's and Bartlett's tests showed, respectively, that the data were neither normal, nor were their variances homogeneous. A non-parametric test would normally be run in this instance, to determine significant difference from control performance. However, in this case, non-parametric tests did not indicate representative results, when compared with the data. Accordingly, the NOAEC (No Observable Adverse Effect Concentration) for survival was determined using analysis of variance followed by Dunnett's test.

3.0 RESULTS AND DISCUSSION

3.1 Microtox Test Results

The 5 and 15-minute EC_{50} values, r^2 values, and 95 percent confidence intervals of the soil extract tests are presented in Table 3-1, and the results of the SPP standard reference toxicant tests are presented in Table 3-2. LTF-E soil was the least toxic, with a 15-minute EC_{50} of 59.1 percent. LTF-D soil was the most toxic, with a 15-minute EC_{50} of 10.1 percent. Raw data sheets are presented in Appendix B.

3.2 Buttercrunch Lettuce (*Lactuca sativa*) Seed Germination Test Results

Seed germination ranged from 10.0 percent in the 100 percent treatment to 86.7 percent in the 12.5 percent treatment (Table 3-3). Consequently, the 5-day EC_{50} for germination was 30 percent test material (Table 3-4). Germination in the control was 93.3 percent. Mean root length ranged from 16.8 mm in the 100 percent treatment to 28.6 mm in the 6.25 percent treatment. Root length (in each of the treatments where germination was not significantly different than control germination) was not significantly different than control root length ($\alpha = 0.05$). Therefore, based on germination, the NOAEC was 12.5 percent test material. Raw data sheets are presented in Appendix C.

3.3 Earthworm Survival Test Results

Earthworm survival ranged from 100 percent in the 6.25 and 12.5 percent treatments to 0 percent in the 100 percent treatment (Table 3-5). Consequently, the 14-day LC_{50} was 27.3 percent test material (Table 3-4). Survival in the control was 100 percent. The NOAEC was 12.5 percent test material. Raw data sheets are presented in Appendix D.

3.4 Analytical Chemistry

Prior to shipping the soil samples to ENSR, a sample was composited by Retec and sent to Alden Analytical Laboratories, Inc. Eighteen organic materials were measured. Phenanthrene was present in the highest concentration - 220,000 $\mu\text{g/kg}$. Other materials present in high concentrations included fluoranthene (100,000 $\mu\text{g/kg}$), acenaphthene (89,000 $\mu\text{g/kg}$), fluorene (74,000 $\mu\text{g/kg}$), and pyrene (67,000 $\mu\text{g/kg}$). A copy of all the data is provided in Appendix E.

TABLE 3-1

**Somers Landfarm Soil Samples
Results of Microtox Analyses**

Sample ID	ENSR Sample Number	Exposure Period (min)	EC ₅₀ (%)	95% Confidence Intervals	R ²
LTF-A	4842 (12384)	5 15	27.3 31.6	21.0 - 36.2 19.3 - 56.7	0.995 0.980
LTF-B	4843 (12385)	5 15	11.6 13.5	6.8 - 20.7 5.6 - 37.0	0.978 0.948
LTF-C	4844 (12386)	5 15	16.3 17.2	13.5 - 19.7 10.3 - 30.0	0.997 0.981
LTF-D	4845 (12387)	5 15	9.8 10.1	1.6 - 93.2 1.4 - 129.1	0.886 0.859
LTF-E	4846 (12388)	5 15	53.0 59.1	29.6 - 123.3 37.9 - 107.0	0.965 0.970
LTF-E (Duplicate)	4846D (12388D)	5 15	57.6 66.2	40.5 - 89.2 38.4 - 155.5	0.986 0.942

TABLE 3-2

Results of Microtox SPP Reference Toxicant Tests

Date of Analysis	Reading Time (minutes)	EC ₅₀ (mg/L)	Historical Mean (mg/L)	Acceptable Range (mg/L)
06/16/93	5	20.3 ¹	14.8	10.5 - 19.0
	15	12.0	9.8	6.6 - 12.9
06/17/93	5	18.4	14.8	10.5 - 19.1
	15	10.0	9.8	6.6 - 12.9

¹ The five minute EC₅₀ of the reference toxicant test conducted on June 16, 1993 indicated the test organisms (*Photobacterium phosphoreum*) were slightly less sensitive to the reference toxicant after five minutes of exposure than what has historically been observed; however, the fifteen minute EC₅₀ was within the sensitivity range. The difference between the five minute EC₅₀ observed on June 16th and the historical sensitivity range was not of significant magnitude to suggest especially tolerant test organisms.

TABLE 3-3

Germination and Root Lengths of *Lactuca sativa* Exposed to
Soil from the Somers Landfarm

Concentration of Test Material (Percent)	Endpoints (Mean Values)	
	Germination (Percent)	Root Lengths (mm)
0 (Control)	93.3	28.9
6.25	76.7	28.6
12.5	86.7	26.2
25	63.3 ¹	24.3
50	23.3 ¹	23.2
100	10.0 ¹	16.8

¹ Indicates a significant difference ($\alpha = 0.05$) from the control, using Dunnett's test.

TABLE 3-4

Median Effective or Lethal Concentration (EC_{50} or LC_{50}) Values for
Lactuca sativa Germination and *Eisenia foetida* Survival

Exposure Period (hours)	EC_{50} or LC_{50} (percent test material)	Statistical Method
5 Days (<i>Lactuca sativa</i>)	30	Spearman-Kärber
14 Days (<i>Eisenia foetida</i>)	27.3	Probit

TABLE 3-5

Survival of *Eisenia foetida* Exposed to
Soil from the Somers Landfarm

Concentration of Test Material (Percent)	Endpoints	
	Survival (Percent)	Number Live Organisms
0 (Control)	100	15
6.25	100	15
12.5	100	15
25	46.7 ¹	7
50	13.3 ¹	2
100	0 ¹	0

¹ Indicates a significant difference ($\alpha = 0.05$) from the control, using Dunnett's test.

4.0 LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating Manual. Microbics Operations. Carlsbad, CA.

USEPA. 1989. Protocols for Short Term Toxicity Screening of Hazardous Waste Sites. EPA/600/3-88/029.

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APPENDIX A
CHAIN OF CUSTODY FORM

CHAIN OF CUSTODY RECORD

Client/Project Name BN - Somers/Galesburg			Project Location Somers, MT			<div style="display: flex; justify-content: space-between;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Micro box</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">Lettuce seed</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">4 bags of manure</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">80X100X100</div> </div>					
Project No. 1140-020-200			Field Logbook No. -								
Samples (Signature) <i>[Signature]</i>			Chain of Custody Tape No. 24178 / 24179								

Sample No./ Identification	Date	Time	Lab Sample Number	Type of Sample	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	85
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8505-093-124-032, 033

APPENDIX B
MICROTOX TEST DATA



ENVIRONMENTAL RESEARCH & TECHNOLOGY, INC.
BOX 2105 1715 HEATH PARKWAY FORT COLLINS COLORADO 80522 (303) 493-8878

DATA PACKAGE FINAL RELEASE FORM

Project Number 1140-020-200
Client ReTec/BN

1. Chain of Custody Records Complete.
2. Sample Log Book "Results Sent" Sign Off Completed.
3. Quality Assurance Data Approval Form Completed.
4. Signature Page Completed.
5. Daily Logs Included and Completed.
6. Serial Dilution Standards Form Included if Applicable.
7. Accuracy Data (Fortified Samples) Included.
8. Precision Data Included.
9. Sample Disposal Considered.
10. Typed Results Checked and Approved.

Checked By	Approved By
SB	dk
SB	dk
SB	dk
SB	dk
SB	dk
N/A	✓
SB	dk
SB	dk
SB	dk
SB	dk

Checked By Susan L. Burnett Approved By Dan Keefe
Date 6/17/93 Date 6/17/93



QUALITY ASSURANCE FORM

CLIENT: ReTec / BN

PROJECT NO: 1140-020-200

ANALYSIS PARAMETER(S): MICROTOX

INCLUDED IN THIS DATA PACKAGE (PLEASE LIST BELOW):

Initial Page(1): Raw Data Sheet(1); Daily Logs(1); Soil Extraction(1);

Standard Dilutions(1); Data Reduction Sheets(1); Charts(1)

ROUTING			SIGNATURE
TITLE	NAME	DATE	DISAPPROVAL (SEE SIDE 2)
INITIATOR	<i>Susan Burnett</i>	<i>6/15/93</i>	
O.A. UNIT	<i>Dan Keefe</i>	<i>6/17/93</i>	

(OVER)

INITIAL PAGE

SIGNATURE

INITIALS

Susan L. Burnett

SB

Susan Keefe

SK

6/31/93

SUBJECT: DAILY LOG DATE AND SIGN EACH ENTRY

6/7/93 Samples received via Fed. Ex. Refrigerated @ 4°C upon
^{SD 413 (S)}
~~rec~~ receipt. (SB)

6/15/93 Started tumbling samples 12384 - 12388D @ 0930. (SB)

6/16/93 Stopped tumbling samples @ 0815 (SB).

6/16/93 Adjusted salinity of 10mL soil extract with 0.2 g NaCl for
each sample, and performed Microtox assays per
Beckman methods. (SB)

sk 6/17/93

ERT

EXTRACTION OF SOIL SAMPLES

Page No.:

Client: ReTec / BN

Project: 1140-020-200

Analysis: MICROTOX

[illegible]

Sh 6/17/53

Comments:**Review By:**

AE 8/31/93

ECKMAN

MICROTOX™ DATA REDUCTION SHEET

INSTRUCTIONS

SPP Standard

ENSR

COLLECTED: _____
DATE: _____
TIME: _____REPORTED RESULTS: TOXIC ☐ NON-TOXIC ☐ MAX ☐ FOR CONCENTRATION: _____RAP ☐CALC ☒ EC _____ MIN _____ CI _____ % SAMPLE _____

CONFIDENCE FACTOR: _____

OTHER _____

95% CONFIDENCE INTERVAL 5min: 17.5 23.6

REMARKS: 15min: 10.7 13.3

SAMPLE DATA: SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): Standard

DATE ASSAYED: 6 - 16 - 93 TIME: 1351

VISUAL COLOR: None CORRECTION REQUIRED: YES ☐ NO ☒VISUAL TURBIDITY: Clear SEPARATION REQUIRED: YES ☐ NO ☒

INITIAL pH: _____ pH ADJUSTED TO: _____ WITH _____

OSMOTICALLY ADJUSTED WITH: MOAS ☐ DRY NaCl ☒ OTHER ☐

PRIMARY DILUTION OF SAMPLE: 100%

OPERATOR: SB REAGENT VIAL LOT NO. M- AM-018

REMARKS: 10ul bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I ₀	FINAL READINGS			BLANK RATIO CHECK	
			5-MIN I ₅	15-MIN I ₁₅	30-MIN I ₃₀	B1: R (5) =	
B1	0 (BLANK)	90	77	65		C1: R (5) =	
C1	0 (BLANK)	84	70	57		ABSORBANCE (COLOR) CORRECTION DATA	
SUMS OF READINGS		A	B	C	D	I ₀ =	
MEAN BLANK RATIOS.		B1 - A = R (5) =		R̄ (5) =	R̄ (15) =	R̄ (30) =	I ₁ =
				0.845			C ₀ =

SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I ₀	FINAL READINGS			Γ (L, T) EFFECTS			
			5-MIN I (5)	15-MIN I (15)	30-MIN I (30)	Γ (5)	Γ (15)	Γ (30)	Γ ² (5)
B2	6.25	93	71	47		0.107			
C2	6.25	93	70	48		0.122			
B3	12.5	93	54	31		0.455			
C3	12.5	98	56	34		0.478			
B4	25.0	89	33	16		1.278			
C4	25.0	92	31	16		1.507			
B5	50.0	84	13	5.6		4.459			
C5	50.0	86	11	5.0		5.605			

FILE IS ST061693
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 16, 1993
ENSR LAB NUMBER: SPP STANDARD
CLIENT ID: NONE

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	20.3	17.5	23.6	0.995
15-MINUTE DATA =	12.0	10.7	13.3	0.996

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	77	65
C1	84	70	57

R (mean)	0.8444	0.7004
R1	0.8556	0.7222
R2	0.8333	0.6786
DIFF	0.0222	0.0437

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	6.25	93	71	47
C2	6.25	93	70	48
B3	12.5	93	54	31
C3	12.5	98	56	34
B4	25	89	33	16
C4	25	92	31	16
B5	50	84	13	5.6
C5	50	86	11	5

PERCENT LIGHT LOSS

	T=5	T=15
	9.6	27.8
	10.9	26.3
	31.2	52.4
	32.3	50.5
	56.1	74.3
	60.1	75.2
	81.7	90.5
	84.9	91.7

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	6.25	0.106	0.386
C2	6.25	0.122	0.357
B3	12.5	0.454	1.101
C3	12.5	0.478	1.019
B4	25	1.277	2.896
C4	25	1.506	3.027
B5	50	4.456	9.506
C5	50	5.602	11.047

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	0.796	-0.974	-0.414
C2	0.796	-0.914	-0.447
B3	1.097	-0.343	0.042
C3	1.097	-0.321	0.008
B4	1.398	0.106	0.462
C4	1.398	0.178	0.481
B5	1.699	0.649	0.978
C5	1.699	0.748	1.043

PREDICTED

CONC	T=5
0.795880	1.510967 0.44
0.795880	1.510967 0.44
1.096910	1.842884 0.74
1.096910	1.842884 0.74
1.397940	2.174802 1.03
1.397940	2.174802 1.03
1.698970	2.506720 1.33
1.698970	2.506720 1.33

ME 8/31/93

FILED _____

5 min: 16.4 20.7

SAMPLE DATA: SAMPLE TYPE EFFLUENT LEACHATE, ETC.) Standard

None

WATER	CLARITY	Clear	TEMPERATURE	55.0	WIND	0.0	WIND DIRECTION	000
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ACTUAL _____ = ADJUSTED TO _____

NOTICIA ADJUSTED WITH GAS BY MICH ☒ OTHER

PRIMARY DILUTION OF SAMPLE. 100%

OPERATOR SB REAGENT VALLEY TWO ~~11~~ AM-018

REMARKS. 10 mL bacteria

BLANK		FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			BLANK RATIO CHECK			
CUVETTE				5 MIN I_{5b}	15-MIN I_{15b}	MIN	B1			
B1	0 (BLANK)		90	79	69		C1			
C1	0 (BLANK)		80	67	59		ABSORBANCE .COLORI CORRECTION DATA			
SUMS OF READINGS							I_0			
MEAN BLANK RATIOS.				$\bar{R} (5) =$	$\bar{R} (15) =$	$\bar{R} ($	$I_f =$			
$\frac{B}{A} = \bar{R} (t) =$							$C_0 =$			
SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING $I(0)$	FINAL READINGS			$I'(t, T)$ EFFECTS				
			5-MIN $I(5)$	15-MIN $I(15)$	MIN	$I'(5)$	$I'(15)$	$I'(\text{---})$	$I'_0(\text{---})$	
B2	6.25	74	54	37						
C2	6.25	76	54	37						
B3	12.5	75	43	25						
C3	12.5	68	38	22						
B4	25.0	63	21	10						
C4	25.0	64	22	10						
B5	50.0	77	11	4.3						
C5	50.0	73	10	4.0						
EC 50 (t-MIN. 15 °C)			BY GRAPH							
			BY CALCULATOR			18.4	10.0			

AE 8/31/93

FILE IS ST061793
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 17, 1993
ENSR LAB NUMBER: SPP STANDARD
CLIENT ID: NONE

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	18.4	16.4	20.7	0.997
15-MINUTE DATA =	10.0	9.0	11.1	0.995

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	79	69
C1	80	67	59
R (mean)	0.8576	0.7521	
R1	0.8778	0.7667	
R2	0.8375	0.7375	
DIFF	0.0403	0.0292	

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	6.25	74	54	37
C2	6.25	76	54	37
B3	12.5	75	43	25
C3	12.5	68	38	22
B4	25	63	21	10
C4	25	64	22	10
B5	50	77	11	4.3
C5	50	73	10	4

PERCENT LIGHT LOSS

T=5	T=15
14.9	33.5
17.2	35.3
33.1	55.7
34.8	57.0
61.1	78.9
59.9	79.2
83.3	92.6
84.0	92.7

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	6.25	0.175	0.504
C2	6.25	0.207	0.545
B3	12.5	0.496	1.256
C3	12.5	0.535	1.325
B4	25	1.573	3.738
C4	25	1.495	3.813
B5	50	5.003	12.468
C5	50	5.261	12.726

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	0.796	-0.756	-0.297
C2	0.796	-0.684	-0.264
B3	1.097	-0.305	0.099
C3	1.097	-0.272	0.122
B4	1.398	0.197	0.573
C4	1.398	0.175	0.581
B5	1.699	0.699	1.096
C5	1.699	0.721	1.105

PREDICTED

CONC	T=5
0.795880	1.578963
0.795880	1.578963
1.096910	1.936599
1.096910	1.936599
1.397940	2.294235
1.397940	2.294235
1.698970	2.651871
1.698970	2.651871

BECKMAN

MICROTOX™ DATA REDUCTION SHEET

AE-8/31/93

12384

LTFA

Retec /BN

REPORTED RESULTS

EC

EXPOSURE TIME

EXPOSURE

5 min:

21.0

36.2

15 min:

19.3

56.7

SAMPLE DATA: SAMPLE TYPE: EFF. DIL. LEACHATE ETC. Soil extract

VOLUME ASSAYED

6

16

93

TIME

1423

SOLVENT

None

CORRECTION

ALL

0.0

SOL CLARITY

Clear

SPAC

0.0

0.0

TEST METHOD

TESTED WITH

X

OTHER

PERCENT DILUTION OF SAMPLE

100%

OPERATOR

SB

REAGENT LOT

X- AM-018

REMARKS

10 uL bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			BLANK RATIO CHECK	
			5 MIN I_5	15-MIN I_{15}	MIN I_∞	B1: I_5/I_0	C1: I_{15}/I_0
B1	(BLANK)	90	77	66			
B2	(BLANK)	84	74	63			
SUMS OF READINGS			ΣI_5	ΣI_{15}	ΣI_∞	I_0	
MEAN BLANK RATIOS			$\bar{I}_5(5) =$	$\bar{I}_{15}(15) =$	$\bar{I}_\infty(5) =$	I_0	
\bar{I}_5	\bar{I}_{15}	$\bar{I}_\infty(t) =$				$C_0 =$	

SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			[t, T] EFFECTS			
			5 MIN I_5	15-MIN I_{15}	MIN I_∞	$I'(5)$	$I'(15)$	$I'(\quad)$	$I_0^*(\quad)$
B2	0.4%	84	71	60					
C2	0.4	87	73	63					
B3	2.0	82	65	57					
C3	2.0	89	70	61					
B4	10.0	79	47	42					
C4	10.0	85	50	45					
B5	50.0	84	27	24					
C5	50.0	89	30	26					
EC _____ (t-MIN, _____ °C)			BY GRAPH						
			BY CALCULATOR			27.3	31.6		

*See Paragraph 11.5 in the Microtox System Operating Manual

r₂: 0.995 0.980

AE-6/17/93

me 8/31/93

FILE IS 12384
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 16, 1993
ENSR LAB NUMBER: 12384
CLIENT ID: LFT-A

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	27.3	21.0	36.2	0.995
15-MINUTE DATA =	31.6	19.3	56.7	0.980

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	77	66
C1	84	74	63

R (mean)	0.8683	0.7417
R1	0.8556	0.7333
R2	0.8810	0.7500
DIFF	-0.0254	-0.0167

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	84	71	60
C2	0.4	87	73	63
B3	2	82	65	57
C3	2	89	70	61
B4	10	79	47	42
C4	10	85	50	45
B5	50	84	27	24
C5	50	89	30	26

PERCENT LIGHT LOSS

T=5	T=15
2.7	3.7
3.4	2.4
8.7	6.3
9.4	7.6
31.5	28.3
32.3	28.6
63.0	61.5
61.2	60.6

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	0.027	0.038
C2	0.4	0.035	0.024
B3	2	0.095	0.067
C3	2	0.104	0.082
B4	10	0.459	0.395
C4	10	0.476	0.401
B5	50	1.701	1.596
C5	50	1.576	1.539

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.565	-1.416
C2	-0.398	-1.459	-1.616
B3	0.301	-1.021	-1.174
C3	0.301	-0.983	-1.086
B4	1.000	-0.338	-0.403
C4	1.000	-0.322	-0.397
B5	1.699	0.231	0.203
C5	1.699	0.198	0.187

PREDICTED
CONC

T=5
-0.39794 0.002339 0.48
-0.39794 0.002339 0.48
0.301029 0.276378 0.62
0.301029 0.276378 0.62
1 0.550417 0.76
1 0.550417 0.76
1.698970 0.824456 0.90
1.698970 0.824456 0.90

At 1/31/93

12385
LTFB
ReTeC / BN

REPORTED RESULTS: _____

EC _____

CONFIDENCE LIMITS _____

CONFIDENCE INTERVAL 5 min: 6.8 20.7

15 min: 5.6 37.0

REMARKS: _____

SAMPLE DATA: SAMPLE TYPE EFFLUENT LEACHATE, ETC. Soil extract

DATE ASSAYED 6 16 93 TIME 1456

APPEARANCE Pale yellow CORRECTION REQUIRED ☐

ODOR Clear SPANATION REQUIRED ☐

INITIAL pH _____

ADJUSTED WITH _____

PRIMARY DILUTION OF SAMPLE 100%

OPERATOR SB REAGENT VIAL LOT NO. AM-018

REMARKS: 10ml bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			BLANK RATIO CHECK			
			5-MIN I_{5b}	15-MIN I_{15b}	_____ MIN $I_{_____b}$	B1: I_0/I_{5b}	B15: I_0/I_{15b}	B()	B()
B1	0 (BLANK)	90	82	71					
C1	0 (BLANK)	87	78	69					
SUMS OF READINGS			ΣB	ΣC	ΣD	ABSORBANCE COLOR CORRECTION DATA			
MEAN BLANK RATIOS			$\bar{R}(5)$	$\bar{R}(15)$	$\bar{R}()$				
$\bar{B} = \bar{A} = \bar{R}(t) =$						I_0	I_{15}	$I()$	$I_0^*()$
SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION: (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			T (t, T) EFFECTS			
			5-MIN $I(5)$	15-MIN $I(15)$	_____ MIN $I()$	$I'(5)$	$I'(15)$	$I'()$	$I_0^*()$
B2	0.4%	87	72	61					
C2	0.4	91	76	67					
B3	2.0	85	65	57					
C3	2.0	84	63	57					
B4	10.0	86	43	41					
C4	10.0	88	46	43					
B5	50.0	87	16	15					
C5	50.0	88	17	16					
EC _____ (t-MIN, _____ °C)			BY GRAPH						
			BY CALCULATOR			11.6	13.5		

AE 8/31/93

FILE IS 12385
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 16, 1993
ENSR LAB NUMBER: 12385
CLIENT ID: LFT-B

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	11.6	6.8	20.7	0.978
15-MINUTE DATA =	13.5	5.6	37.0	0.948

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	82	71
C1	87	78	69
R (mean)	0.9038	0.7910	
R1	0.9111	0.7889	
R2	0.8966	0.7931	
DIFF	0.0146	-0.0042	

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	87	72	61
C2	0.4	91	76	67
B3	2	85	65	57
C3	2	84	63	57
B4	10	86	43	41
C4	10	88	46	43
B5	50	87	16	15
C5	50	88	17	16

PERCENT LIGHT LOSS

T=5	T=15
8.4	11.4
7.6	6.9
15.4	15.2
17.0	14.2
44.7	39.7
42.2	38.2
79.7	78.2
78.6	77.0

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	-0.092	0.128
C2	0.4	0.082	0.074
B3	2	0.182	0.180
C3	2	0.205	0.166
B4	10	0.808	0.659
C4	10	0.729	0.619
B5	50	3.915	3.588
C5	50	3.679	3.350

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.036	-0.892
C2	-0.398	-1.085	-1.129
B3	0.301	-0.740	-0.746
C3	0.301	-0.688	-0.781
B4	1.000	-0.093	-0.181
C4	1.000	-0.137	-0.208
B5	1.699	0.593	0.555
C5	1.699	0.566	0.525

PREDICTED

CONC	T=5
-0.39794	-0.24235 0.13
-0.39794	-0.24235 0.13
0.301029	0.461479 0.52
0.301029	0.461479 0.52
1	1.165311 0.90
1	1.165311 0.90
1.698970	1.869142 1.29
1.698970	1.869142 1.29

BECKMAN

MICROTOX™ DATA REDUCTION SHEET

AE-8/31/93

12386
LTC
ReTec / BN

TESTED BY
DATE
TIME

REPORTED RESULTS

EC

CONFIDENCE FACTOR

CONFIDENCE INTERVAL 5 min: 13.5 19.7
15 min: 10.3 30.0

REMARKS

SAMPLE DATA: SAMPLE TYPE REFERENCE MATERIAL ETC. Soil extract

DATE ASSAYED 6 16 93 TIME 1527

ORIGINAL COLOR Pale yellow CORRECTION REQUIRED YES NO

ORIGINAL TURBIDITY Clear SEPARATION REQUIRED YES NO

TESTAL VIAL

WATERBATH COATED WITH OTHER

PRIMARY DILUTION OF SAMPLE 100%

OPERATOR SB REAGENT VIAL LOT NO AM-018

REMARKS 10 mL bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I_{0b}	5 MIN I_{5b}	15-MIN I_{15b}	MIN	BLANK RATIO CHECK
B1	0 (BLANK)	90	82	73		B1: 1.0
C1	0 (BLANK)	91	82	74		C1: 1.0
SUMS OF READINGS			ΣB	ΣC	ΣD	I_0
MEAN BLANK RATIOS			$\bar{R}(5)$	$\bar{R}(15)$	$\bar{R}(t)$	I_t
$\bar{B} = \frac{\Sigma B}{n}$			$\bar{R}(t) = \frac{\bar{R}(5) + \bar{R}(15) + \bar{R}(t)}{3}$			$C_0 =$
SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING $I(0)$	5-MIN $I(5)$	15-MIN $I(15)$	MIN	(t, T) EFFECTS
B2	0.4%	87	75	65		$I'(5)$ $I'(15)$ $I'(t)$ $I'_0(t)$
C2	0.4	82	71	62		
B3	2.0	83	65	58		
C3	2.0	82	64	55		
B4	10.0	84	47	43		
C4	10.0	85	47	44		
B5	50.0	87	21	20		
C5	50.0	82	20	19		
EC (t-MIN. °C)			BY GRAPH			
			BY CALCULATOR			16.3 17.2

FILE IS 12386
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

RE 8/31/93

DATE OF ANALYSIS: JUNE 16, 1993
ENSR LAB NUMBER: 12386
CLIENT ID: LFT-C

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	16.3	13.5	19.7	0.997
15-MINUTE DATA =	17.2	10.3	30.0	0.981

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	82	73
C1	91	82	74
R (mean)	0.9061	0.8121	
R1	0.9111	0.8111	
R2	0.9011	0.8132	
DIFF	0.0100	-0.0021	

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	87	75	65
C2	0.4	82	71	62
B3	2	83	65	58
C3	2	82	64	55
B4	10	84	47	43
C4	10	85	47	44
B5	50	87	21	20
C5	50	82	20	19

PERCENT LIGHT LOSS

T=5	T=15
4.9	8.0
4.4	6.9
13.6	14.0
13.9	17.4
38.2	37.0
39.0	36.3
73.4	71.7
73.1	71.5

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	0.051	0.087
C2	0.4	0.046	0.074
B3	2	0.157	0.162
C3	2	0.161	0.211
B4	10	0.619	0.587
C4	10	0.639	0.569
B5	50	2.754	2.533
C5	50	2.715	2.505

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.292	-1.060
C2	-0.398	-1.333	-1.130
B3	0.301	-0.804	-0.790
C3	0.301	-0.793	-0.676
B4	1.000	-0.208	-0.232
C4	1.000	-0.195	-0.245
B5	1.699	0.440	0.404
C5	1.699	0.434	0.399

PREDICTED

CONC	T=5
-0.39794	-0.13908 0.26
-0.39794	-0.13908 0.26
0.301029	0.383360 0.54
0.301029	0.383360 0.54
1	0.905805 0.82
1	0.905805 0.82
1.698970	1.428249 1.10
1.698970	1.428249 1.10

BECKMAN

MICROTOX™ DATA REDUCTION SHEET

EXPERIMENT NO. 12387

LTFD

AE 8131/93

NAME ReTec / BN

COLLECTED BY

DATE

TIME

REPORTED RESULTS:

APP

EC

CONFIDENCE INTERVAL

CONFIDENCE INTERVAL

5 min:

1.6

93.2

15 min:

1.4

129.1

REMARKS:

SAMPLE DATA: SAMPLE TYPE EFFLUENT LEACHATE, ETC. Soil extract

DATE ASSAYED

6

16

93

TIME

1600

VISUAL COLOR

Pale Yellow

CORRECTION REQUIRED

YES ☐

NO ☐

VISUAL TURBIDITY

Clear

SEPARATION REQUIRED

YES ☐

NO ☐

INTERFERENCE

INDICATED BY

NOTICABLY ADJUSTED WITH

PH

BY

X

OTHER

PRIMARY DILUTION OF SAMPLE

100%

OPERATOR

SB

REAGENT VIAL LOT NO.

AM-018

REMARKS:

10 mL bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE		FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			BLANK RATIO CHECK			
				5-MIN I_5	15-MIN I_{15}	MIN I_∞	B1: $R(5)$ _____ C1: $R(5)$ _____			
B1		0 (BLANK)	90	82	72					
C1		0 (BLANK)	95	83	75		ABSORBANCE (COLOR) CORRECTION DATA			
SUMS OF READINGS			ΣA	ΣB	ΣC		$I_0 =$ _____ $I_r =$ _____ $C_0 =$ _____			
MEAN BLANK RATIOS.			$\bar{R}(5) =$ _____ $\bar{R}(15) =$ _____ $\bar{R}(\infty) =$ _____							
$\frac{B}{A} = \frac{C}{A} = \bar{R}(t) =$										
SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			$I(t, T)$ EFFECTS				
			5-MIN $I(5)$	15-MIN $I(15)$	MIN $I(\infty)$	$I(5)$	$I(15)$	$I(\infty)$	I_0°	
B2	0.4%	89	78	69						
C2	0.4	88	66	56						
B3	2.0	82	62	55						
C3	2.0	84	63	58						
B4	10.0	84	39	36						
C4	10.0	82	39	36						
B5	50.0	87	12	11						
C5	50.0	85	12	11						
EC _____ (t-MIN, _____ °C)			BY GRAPH							
			BY CALCULATOR			9.8	10.1			

*See Paragraph 11.5 in the Microtox System Operating Manual

RA = 0.986

0.850 at 1.0103

FILE IS 12387
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

re 8/31/93

DATE OF ANALYSIS: JUNE 16, 1993
ENSR LAB NUMBER: 12387
CLIENT ID: LFT-D

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	9.8	1.6	93.2	0.886
15-MINUTE DATA =	10.1	1.4	129.1	0.859

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	82	72
C1	95	83	75

R (mean)	0.8924	0.7947
R1	0.9111	0.8000
R2	0.8737	0.7895
DIFF	0.0374	0.0105

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	89	78	69
C2	0.4	88	66	56
B3	2	82	62	55
C3	2	84	63	58
B4	10	84	39	36
C4	10	82	39	36
B5	50	87	12	11
C5	50	85	12	11

PERCENT LIGHT LOSS

T=5	T=15
1.8	2.4
16.0	19.9
15.3	15.6
16.0	13.1
48.0	46.1
46.7	44.8
84.5	84.1
84.2	83.7

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	0.018	0.025
C2	0.4	0.190	0.249
B3	2	0.180	0.185
C3	2	0.190	0.151
B4	10	0.922	0.854
C4	10	0.876	0.810
B5	50	5.470	5.286
C5	50	5.321	5.141

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.739	-1.600
C2	-0.398	-0.722	-0.604
B3	0.301	-0.744	-0.733
C3	0.301	-0.722	-0.821
B4	1.000	-0.035	-0.068
C4	1.000	-0.057	-0.091
B5	1.699	0.738	0.723
C5	1.699	0.726	0.711

PREDICTED
CONC

T=5	T=15
-0.39794	-0.34058
-0.39794	-0.34058
0.301029	0.535788
0.301029	0.535788
1	1.412158
1	1.412158
1.698970	2.288529
1.698970	2.288529

BECKMAN

MICROTOX DATA REDUCTION SHEET

AE 8/31/93

12388

LFT-E

ReTeC / BN

REPORTED RESULTS:

EC

CONFIDENCE FACTOR

CONFIDENCE INTERVAL

5 min: 29.6 123.3
15 min: 37.9 107.0

SAMPLE DATA: SAMPLE TYPE EFFLUENT LEACHATE, ETC.

soil extract

DATE ASSAYED: 6 17 93

TIME: 1058

APPEARANCE: Light yellow

CORRECTION REQUIRED: NO

USUAL TURBIDITY: Clear

SEPARATION REQUIRED: NO

STRAIN

PHOTOMETRICALLY ADJUSTED WITH

WAS

EX

TEMP

PRIMARY DILUTION OF SAMPLE:

100%

OPERATOR: SB

REAGENT LOT NO

AM-018

REMARKS:

10 uL bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			BLANK RATIO CHECK			
			5 MIN I_{5b}	15-MIN I_{15b}	MIN $I_{\infty b}$	B1: (5)	B1: (15)	C1: (5)	C1: (15)
B1	0 (BLANK)	90	80	71					
C1	0 (BLANK)	89	78	69					
SUMS OF READINGS			ΣI_{5b}	ΣI_{15b}	$\Sigma I_{\infty b}$	ABSORBANCE COLOR CORRECTION DATA			
MEAN BLANK RATIOS:			$\bar{R}(5)$	$\bar{R}(15)$	$\bar{R}(\infty)$	$I_0 =$	$I_5 =$	$I_{15} =$	$I_{\infty} =$
$\bar{B} = \frac{A}{I_0} = \bar{R}(t) =$									
SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING I_0	FINAL READINGS			$I(t, T)$ EFFECTS			
			5-MIN $I(5)$	15-MIN $I(15)$	MIN $I(\infty)$	$I(5)$	$I(15)$	$I(\infty)$	I_0^*
B2	0.4%	85	74	64					
C2	0.4	83	71	62					
B3	2.0	85	71	61					
C3	2.0	84	71	60					
B4	10.0	83	60	53					
C4	10.0	86	61	54					
B5	50.0	87	36	32					
C5	50.0	86	37	32					
EC (t-MIN, °C)			BY GRAPH						
			BY CALCULATOR			53.0	59.1	Ak 6/1/93	

FILE IS 12388
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 17, 1993
ENSR LAB NUMBER: 12388
CLIENT ID: LFT-E

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	53.0	29.6	123.3	0.965
15-MINUTE DATA =	59.1	37.9	107.0	0.970

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	80	71
C1	89	78	69
R (mean)	0.8826	0.7821	
R1	0.8889	0.7889	
R2	0.8764	0.7753	
DIFF	0.0125	0.0136	

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	85	74	64
C2	0.4	83	71	62
B3	2	85	71	61
C3	2	84	71	60
B4	10	83	60	53
C4	10	86	61	54
B5	50	87	36	32
C5	50	86	37	32

PERCENT LIGHT LOSS

T=5	T=15
1.4	3.7
3.1	4.5
5.4	8.2
4.2	8.7
18.1	18.4
19.6	19.7
53.1	53.0
51.3	52.4

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	0.014	0.039
C2	0.4	0.032	0.047
B3	2	0.057	0.090
C3	2	0.044	0.095
B4	10	0.221	0.225
C4	10	0.244	0.246
B5	50	1.133	1.126
C5	50	1.052	1.102

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.859	-1.412
C2	-0.398	-1.497	-1.328
B3	0.301	-1.246	-1.047
C3	0.301	-1.354	-1.023
B4	1.000	-0.656	-0.648
C4	1.000	-0.612	-0.610
B5	1.699	0.054	0.052
C5	1.699	0.022	0.042

PREDICTED
CONC

T=5
-0.39794 0.121675 0.54
-0.39794 0.121675 0.54
0.301029 0.186103 0.57
0.301029 0.186103 0.57
1 0.250532 0.61
1 0.250532 0.61
1.698970 0.314960 0.64
1.698970 0.314960 0.64

BIOKMAN

BIOKMAN DATA REDUCTION SHEET

12388D

LFT-E

AE 8/31/93

ReTec / BN

TESTED RESULTS

EC

5 min: 40.5
15 min: 38.489.2
155.5

SAMPLE DATA

Soil extract

NO. SAYED

6

17

93

1134

Light Yellow
Clear

X

100%

OPERATOR

SB

REAGENT

AM-018

REMARKS

10mL bacteria

TABLE OF OBSERVED AND CALCULATED RESULTS

BLANK CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING (10)	FINAL READINGS			BLANK RATIO CHECK	
			5 MIN (5)	15 MIN (15)	MIN ()	B1	B2
1	BLANK	90	82	73		C1	
2	BLANK	91	84	72		ABSORBANCE (COLOR) CORRECTION DATA	
AVERAGE OF READINGS			81	71		C3	
MEAN BLANK RATIOS			B (5) = B (15) = B ()			C4	
1	A	R (1)				C5	

SAMPLE CUVETTE	FINAL ASSAY CONCENTRATION (SPECIFY UNITS)	INITIAL READING (10)	FINAL READINGS			(1, 15) EFFECTS			
			5 MIN (5)	15 MIN (15)	MIN ()	(5)	(15)	()	Γ ₀ ()
B2	0.4%	87	78	68					
C2	0.4	91	82	71					
B3	2.0	95	82	73					
C3	2.0	89	78	69					
B4	10.0	94	71	62					
C4	10.0	94	71	63					
B5	50.0	91	41	36					
C5	50.0	85	39	34					
EC (t-MIN. °C)			BY GRAPH						
			BY CALCULATOR			57.6	66.2		AE 8/31/93

AE 8/31/93

FILE IS 12388D
MICROTOX CALCULATION SPREADSHEET
DATA FOR 5 AND 15 MINUTES

DATE OF ANALYSIS: JUNE 17, 1993
ENSR LAB NUMBER: 12388D
CLIENT ID: LFT-E

	EC50	LOWER CI	UPPER CI	R2
5-MINUTE DATA =	57.6	40.5	89.2	0.986
15-MINUTE DATA =	66.2	38.4	155.5	0.942

BLANK READINGS

CUVETTE	T=0	T=5	T=15
B1	90	82	73
C1	91	84	72
R (mean)		0.9171	0.8012
R1		0.9111	0.8111
R2		0.9231	0.7912
DIFF		-0.0120	0.0199

SAMPLE READINGS

CUVETTE	CONC	T=0	T=5	T=15
B2	0.4	87	78	68
C2	0.4	91	82	71
B3	2	95	82	73
C3	2	89	78	69
B4	10	94	71	62
C4	10	94	71	63
B5	50	91	41	36
C5	50	85	39	34

PERCENT LIGHT LOSS

T=5	T=15
2.2	2.4
1.7	2.6
5.9	4.1
4.4	3.2
17.6	17.7
17.6	16.3
50.9	50.6
50.0	50.1

GAMMA CALCULATIONS

CUVETTE	CONC	T=5	T=15
B2	0.4	-0.023	0.025
C2	0.4	0.018	0.027
B3	2	0.062	0.043
C3	2	0.046	0.033
B4	10	0.214	0.215
C4	10	0.214	0.195
B5	50	1.036	1.025
C5	50	0.999	1.003

LOG TRANSFORMATIONS

CUVETTE	CONC	T=5	T=15
B2	-0.398	-1.640	-1.602
C2	-0.398	-1.751	-1.571
B3	0.301	-1.204	-1.371
C3	0.301	-1.333	-1.477
B4	1.000	-0.669	-0.668
C4	1.000	-0.669	-0.709
B5	1.699	0.015	0.011
C5	1.699	-0.001	0.001

PREDICTED

CONC	T=5	T=15
-0.39794	0.148113	0.63
-0.39794	0.148113	0.63
0.301029	0.166104	0.64
0.301029	0.166104	0.64
1	0.184096	0.64
1	0.184096	0.64
1.698970	0.202088	0.65
1.698970	0.202088	0.65

APPENDIX C
LETTUCE SEED GERMINATION TEST DATA

TOXICITY DATA PACKAGE COVER SHEET

Test Type: Acute (Sub)Chronic
Test Substance: Effluent Other: Sediment
Dilution Water: Receiving Receiving Match Effluent Match
Mod. Hard Hard Very Hard Other (Specify): Sand
Dilution Water RW# or ENSR#: 4842-
FCETL Sample Number: 4846 Sample Type: Composite
Collection Date and Time: From: 6/13/93 @ @ @
To: @ @ @
Date and Time Test Began: 6/16/93 @ 1630 Date and Time Test Ended: 6/21/93 @ 1530
Protocol Number: Investigator(s): DAP

Background Information

Type of Test: Lettuce Seed Germination pH Control?: Yes No If Yes, give % CO₂:
Test Temperature: 25°C Test Chambers: 100 x 15 mm Petridishes
Test Solution Vol.: 50g Number of Replicates per Treatment: 3
Length of Test: 5 days Number of Organisms per Replicate: 10
Type of Food and Quantity per Chamber: NA Feeding Frequency: NA
Test Substance Characterization Parameters and Frequency:
NH₃: NA pH: Hardness: NA Alkalinity: NA
Conductivity: NA TRC: NA

Test Concentrations (Volume:Volume): 100, 50, 25, 12.5, 6.25, 0
Agency Summary Sheet(s)?: None Yes (Specify):

Reference Toxicant Data:	Test Dates: <u> </u> to <u> </u>	LC ₅₀ or IC ₂₅ (Circle): <u> </u>
Hist. 95% Confid. Inter.:	<u> </u> to <u> </u>	Method for Determining Ref. Tox. Value: <u> </u>

Special Procedures and Considerations: 5 soil samples composited + 40 test
run.

Study Director Initials: DAP Date: 6/16/93

SEED GERMINATION BIOLOGICAL DATA

Project Number: <u>505-073-124-032</u>
Test Species (Circle) <u>Lactuca sativa</u> Other (Specify):

Conc.	Test Replicate	Initial Number of Seeds	Number of Germinated Seeds	Remarks
0	A	10	10	
	B	10	9	
	C	10	?	
	D			
6.25	A	10	8	
	B	10	7	
	C	10	8	
	D			
12.5	A	10	9	
	B	10	7	
	C	10	10	
	D			
25	A	10	8	
	B	10	6	
	C	10	5	
	D			
50	A	10	3	
	B	10	0	
	C	10	4	
	D			
100	A	10	2	
	B	10	1	
	C	10	0	
	D			
	Date:	6/16/93	6/21/93	
	Time:	1630	1530	
	Initials:	DAP	DAP	

ROOT ELONGATION PHYTOTOXICITY

FCETL QA Form No. 068
Effective 8/91
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Client: <u>BN</u>			Project #: <u>8505-093-124-032</u>			Sample I.D.:		
Date Received: <u>6/7/93</u>			Date Test Initiated: <u>6/16/93</u>			Date Test Terminated: <u>6/21/93</u>		
Date Elutriate Prepared: <u>NA</u>			Time Initiated: <u>1630</u>			Time Terminated: <u>1530</u>		
Seed: <u>Lactuca sativa</u>			Seed Size Grade: <u>#3</u>			Tech(s): <u>DAP</u>		

Sample Type ¹	Conc.	Rep	Root Length (mm)			Comments	Init.	Hardness (mg/L CaCO ₃)	Alkalinity (mg/L CaCO ₃)	Initial pH	Final pH	
NC	0	A	39 24	28 27	35 24	20 27	35 30	$\bar{X} = 28.9$	DAP	NA	NA	NA
↓	↓	B	37 35	20 44	40 30	37 20	30	32.6				
↓	↓	C	29 36	24 22	30 30	29 3	24	25.2				
S	6.25	A	21 35	28 15	41 27	24 40		28.9				
↓	↓	B	28 10	26 46	46 32	17		29.6				
↓	↓	C	30 22	32 42	24 33	24 12		27.4				
↓	12.5	A	44 20	26 36	2 15	35 40	40	28.7				
↓	↓	B	10 20	26 22	15 26	26		20.7				
↓	↓	C	33 35	30 26	42 31	35 20	24 10	29.2				
↓	25	A	36 27	26 32	33 25	20 16		26.9				
↓	↓	B	13 10	14 35	37 20			21.5				
↓	↓	C	37 25	15 25	20			24.4				
↓	50	A	22 23	35				26.7				
↓	↓	B						No germination (0)				
↓	↓	C	21 15	28 15				19.6				

¹ E.g., Sample (S), Positive Control (PC), Negative Control (NC)

an 8/20/13

Effective 8/91

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¹ E.g., Sample (S), Positive Control (PC), Negative Control (NC)

DAF 8/27/73

7. 8/31/13

Hydration Water Volumes per Concentration

[illegible]¹ Amount of Hydration Water per Test Chamber = (A + 100) x B



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FCETL QA Form No. 071
Effective 11/91

04p 8/27/23
At 8/31/23

Moisture Fraction Calculation

Sponsor/Client: <u>Retec / BN</u>			Test Substance: <u>Soil</u>			Date Received: <u>6/7/93</u>			Date(s) Homogenized: <u>6/14/93</u>		
Project No: <u>1140-020-200</u>			Date Sampled: <u>6/3/93</u>			Date In Oven: <u>6/14/93</u> Time In: <u>1600</u>			Date Out: <u>6/15/93</u> Time Out: <u>1430</u>		
Sample Number	ENSR Number	Container Size	Boat Weight (g)	Weight of Soil (g) (A)	Total Initial Wet Wt. (Soil + Boat) (g) (B)	Total Final Dry Weight (g) (C)	Moisture Fraction (MF) (E)	Amount of Wet Soil to Tumble	Amount of MQ Water to Tumble	Date/Time Tumbling Began	Date/Time Tumbling Ended
<u>LTFA, LTFB</u>	<u>4842, 4843</u>		<u>247.3g</u>	<u>196.6</u>	<u>443.9</u>	<u>386.9</u>	<u>13.7%</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>
<u>LTFC</u>	<u>4844</u>										
<u>LTFD</u>	<u>4845</u>										
<u>LTFE</u>	<u>4846</u>										
<u>(Combined)</u>	<u>Combined</u>										

$$\text{Moisture Fraction} = (B-C)/A$$

Quantity of Wet Sediment to Tumble = 75g + E(75)
Quantity of Milli-Q Water to Tumble = 300 mL - E(75)



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FCETL QA Form No. 072
Effective 11/91

DAP 8/27/93
AE 8/31/93

Water Holding Capacity

Sponsor/Client: Rotec/BN		Test Substance: Soil			Date Received: 6/7/93				
Project No.:		Date Sampled: 6/3/93			Today's Date: 6/15/93				
Sample Number	ENSR Number	Weight of Dry Sample (g) ¹ (A)	Amount of MQ or DI Water Added (mL)	Wt. of Funnel & Wetted Filter Paper (g) (B)	Time Start Drain	Time End Drain	Final Weight of Funnel, Paper, & Soil (g) (C)	WHC (mL) per 100 g (A) of Dried Sample	Remarks
LTFA-	4842-	116.8	100+	167.5	1512	1745	325	34.8	17.4 mL/50 g soil
LTFE	4846								
combined	combined								

¹Dried at 104°C for 24 hours.

$$\text{WHC} = \text{C} \div (\text{A} + \text{B})$$

If more or less than 100 g of dried soil was used, then: $\text{WHC per 100g} = 100 \div \text{A}(\text{WHC})$

9 of 15
DAP 8/27/93
AE 8/31/93

CHEMICAL: SEDIMENT
va

SPECIES: Lataca sat:

RAW DATA:

CONCENTRATION(%)	6.25	12.50	25.00	50.00	100.00
NUMBER EXPOSED:	30	30	30	30	30
MORTALITIES:	7	4	11	23	27
SPEARMAN-KARBER TRIM:	18.33%				

SPEARMAN-KARBER ESTIMATES:	LC50:	30.04
95% LOWER CONFIDENCE:		23.43
95% UPPER CONFIDENCE:		38.50

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

NOTE: REQUESTED TRIM OF 0.00% IS TOO SMALL.
CALCULATED TRIM OF 18.33% WAS USED.

WOULD YOU LIKE TO HAVE A COPY SENT TO THE PRINTER(Y/N)?

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 8/27/93
CHEMICAL: SEDIMENT

TEST NUMBER: 124-032

DURATION: 5 DAYS
SPECIES: Lataca sat

RAW DATA:

CONCENTRATION(%)	6.25	12.50	25.00	50.00	100.00
NUMBER EXPOSED:	30	30	30	30	30
MORTALITIES:	7	4	11	23	27
SPEARMAN-KARBER TRIM:	18.33%				

SPEARMAN-KARBER ESTIMATES:	LC50:	30.04
95% LOWER CONFIDENCE:		23.43
95% UPPER CONFIDENCE:		38.50

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

NOTE: REQUESTED TRIM OF 0.00% IS TOO SMALL.
CALCULATED TRIM OF 18.33% WAS USED.

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QAP 8/27/93
AE 8/31/93

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.372

W = 0.965

Critical W (P = 0.05) (n = 18) = 0.897

Critical W (P = 0.01) (n = 18) = 0.858

Data PASS normality test at P=0.01 level. Continue analysis.

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.89

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

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DAP 8/27/93
AE 8/31/93

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	1.249	1.412	1.303
2	6.25	3	0.991	1.107	1.068
3	12.5	3	0.991	1.412	1.217
4	25	3	0.785	1.107	0.926
5	50	3	0.159	0.685	0.474
6	100	3	0.159	0.464	0.315

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.009	0.094	0.054
2	6.25	0.004	0.067	0.039
3	12.5	0.045	0.212	0.123
4	25	0.027	0.165	0.095
5	50	0.077	0.278	0.161
6	100	0.023	0.153	0.088

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.444	0.489	15.752
Within (Error)	12	0.372	0.031	
Total	17	2.817		

Critical F value = 3.11 (0.05,5,12)

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

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NAP 5/27/93
AE 8/31/93

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.303	0.933		
2	6.25	1.068	0.767	1.633	
3	12.5	1.217	0.867	0.598	
4	25	0.926	0.633	2.622	*
5	50	0.474	0.233	5.764	*
6	100	0.315	0.100	6.873	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

8505-093-124-032 L. sativa germination test

File: a:\124.32g Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	6.25	3	0.274	29.4	0.167
3	12.5	3	0.274	29.4	0.067
4	25	3	0.274	29.4	0.300
5	50	3	0.274	29.4	0.700
6	100	3	0.274	29.4	0.833

13 JF 15

OAP 8/27/93

AE 8/31/93

8505-093-124-032 root length test

File: a:\124.32r Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 75.407

W = 0.940

Critical W (P = 0.05) (n = 9) = 0.829

Critical W (P = 0.01) (n = 9) = 0.764

Data PASS normality test at P=0.01 level. Continue analysis.

8505-093-124-032 root length test

File: a:\124.32r Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.65

Table Chi-square value = 9.21 (alpha = 0.01)

Table Chi-square value = 5.99 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00

Used for Chi-square table value ==> df (#groups-1) = 2

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

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DAP 8/27/93

AE 8/31/93

8505-093-124-032 root length test

File: a:\124.32r

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	25.200	32.600	28.900
2	6.25	3	27.400	29.600	28.633
3	12.5	3	20.700	29.200	26.200

8505-093-124-032 root length test

File: a:\124.32r

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	13.690	3.700	2.136
2	6.25	1.263	1.124	0.649
3	12.5	22.750	4.770	2.754

8505-093-124-032 root length test

File: a:\124.32r

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	2	13.282	6.641	0.528
Within (Error)	6	75.407	12.568	
Total	8	88.689		

Critical F value = 5.14 (0.05,2,6)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

8505-093-124-032 root length test

File: a:\124.32r

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	28.900	28.900		

150F15
DAP 8/27/93
AE 8/31/93

2	6.25	28.633	28.633	0.092
3	12.5	26.200	26.200	0.933

Dunnett table value = 2.34 (1 Tailed Value, P=0.05, df=6,2)

8505-093-124-032 root length test

File: a:\124.32r Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	6.25	3	6.773	23.4	0.267
3	12.5	3	6.773	23.4	2.700

8505-093-124-032, 033

APPENDIX D
EARTHWORM SURVIVAL TEST DATA

DAP 8/27/93
AE 8/31/93

EARTHWORM SURVIVAL

FCETL QA Form No. 075

Effective 10/91

Page 1 of 6

Client: <u>Rotec / BN</u>		Project Number: <u>8505-073-124</u>		Sample Number: <u>LTFX - LTFE²</u>				
Date Received: <u>6/17/93</u>		ENSR Number: <u>4842-4846²</u>		No. per Replicate: <u>5 worms</u>				
Date Test Initiated: <u>6/29/93</u>		Time Initiated: <u>1700</u>		Techs: <u>DLP SP</u>				
Date Test Terminated: <u>7/13/93</u>		Time Terminated: <u>1530</u>						
Total Initial Worm Wt./No. of Worms Weighed: <u>3.04g/10</u>				Species: <u>Eisenia Foetida</u>				
Sample Type ¹	Conc.	Rep.	No. of Surviving Worms	Total Wt. per Rep. (g)	Mean Wt. per Worm (g)	pH Init/Final	Comments	Init.
<u>1/C</u>	<u>0</u>	<u>A</u>	<u>5</u>	<u>1.36</u>	<u>0.27</u>	<u>6.8/7.7</u>	<u>Caco's added 15ml/200g soil</u>	
		<u>B</u>	<u>5</u>	<u>1.24</u>	<u>0.25</u>			
		<u>C</u>	<u>5</u>	<u>1.29</u>	<u>0.26</u>			
<u>6.25</u>	<u>6.25</u>	<u>A</u>	<u>5</u>	<u>1.41</u>	<u>0.28</u>			
		<u>B</u>	<u>5</u>	<u>1.07</u>	<u>0.21</u>			
		<u>C</u>	<u>5</u>	<u>1.40</u>	<u>0.28</u>			
	<u>12.5</u>	<u>A</u>	<u>5</u>	<u>1.41</u>	<u>0.28</u>			
		<u>B</u>	<u>5</u>	<u>1.42</u>	<u>0.28</u>			
		<u>C</u>	<u>5</u>	<u>1.31</u>	<u>0.26</u>			
	<u>25</u>	<u>A</u>	<u>4</u>	<u>1.30</u>	<u>0.32</u>			
		<u>B</u>	<u>1</u>	<u>0.33</u>	<u>0.33</u>			
		<u>C</u>	<u>2</u>	<u>0.42</u>	<u>0.21</u>			
	<u>50</u>	<u>A</u>	<u>0</u>	<u>—</u>	<u>—</u>			
		<u>B</u>	<u>2</u>	<u>0.48</u>	<u>0.24</u>			
		<u>C</u>	<u>0</u>	<u>—</u>	<u>—</u>			
	<u>100</u>	<u>A</u>	<u>0</u>	<u>—</u>	<u>—</u>	<u>8.3/8.1</u>	<u>Temperatures measured at initiation after worms were in soil for 24 hours</u>	
		<u>B</u>	<u>0</u>	<u>—</u>	<u>—</u>		<u>Clitella worms in 100% were "ballooned" greatly expanded.</u>	
		<u>C</u>	<u>0</u>	<u>—</u>	<u>—</u>			

27°C

28°C
Term.

27°C a
Init

27°C (a)
Term.

¹ E.g., Sample (S), Positive Control (PC), Negative Control (NC)

² Combined 5 samples

8505-093-124-033 Earthworm Survival
Program run on 07-27-1993 at 09:42:46

2 of 5
ODP 8/27/93
AE 8/31/93

RESULTS CALCULATED USING THE BINOMIAL METHOD

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	15	15	100.00	0.0031
50	15	13	86.67	0.3693
25	15	8	53.33	50.0000
12.5	15	0	0.00	0.0031
6.25	15	0	0.00	0.0031

THE BINOMIAL TEST SHOWS THAT 12.5 AND 50 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT

AN APPROXIMATE LC50 OF 24.22583 IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN 12.5 AND 25

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	0.071	28.466	22.239	37.172
3	0.071	28.466	22.386	35.013
2	0.115	26.863	21.809	33.708

AN LC50 CALCULATED USING THE MOVING AVERAGE METHOD MAY NOT
BE A VERY GOOD ESTIMATE IF THE SPAN IS MUCH LESS THAN THE
NUMBER OF CONCENTRATIONS.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	CHI-SQUARE	GOODNESS OF FIT PROBABILITY
5	0.183	1.000	1.867	0.600

SLOPE = 5.239575
95 PERCENT CONFIDENCE LIMITS = 3.000567 AND 7.478582

LC50 = 27.29226
95 PERCENT CONFIDENCE LIMITS = 21.68397 AND 34.34245

LC1 = 9.818682
95 PERCENT CONFIDENCE LIMITS = 4.365329 AND 13.98314

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE
REASONABLE.

8505-093-124
File: a:\124ew

SURVIVAL

Transform: ARC SINE(SQUARE ROOT(Y))

3 of 5
DAP 8/27/93
AE 8/31/93

Shapiro Wilks test for normality

D = 0.354

W = 0.753

Critical W (P = 0.05) (n = 18) = 0.897

Critical W (P = 0.01) (n = 18) = 0.858

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance

Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	1.345	1.345	1.345
2	6.25	3	1.345	1.345	1.345
3	12.5	3	1.345	1.345	1.345
4	25	3	0.464	1.107	0.752
5	50	3	0.226	0.685	0.379
6	100	3	0.226	0.226	0.226

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

4 of 5
QAP 8/27/93
AE 8/31/93

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.000	0.000	0.000
2	6.25	0.000	0.000	0.000
3	12.5	0.000	0.000	0.000
4	25	0.107	0.327	0.189
5	50	0.070	0.265	0.153
6	100	0.000	0.000	0.000

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4.031	0.806	26.867
Within (Error)	12	0.354	0.030	
Total	17	4.385		

Critical F value = 3.11 (0.05,5,12)

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	1.345	1.000		
2	6.25	1.345	1.000	0.000	
3	12.5	1.345	1.000	0.000	
4	25	0.752	0.467	4.196	*
5	50	0.379	0.133	6.836	*
6	100	0.226	0.000	7.918	*

Dunnett table value = 2.50 (1 Tailed Value, $P=0.05$, $df=12,5$)

8505-093-124

File: a:\124ew

Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2

H_0 : Control < Treatment

5 of 5

DAP 5/2/93

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	3			
2	6.25	3	0.249	24.9	0.000
3	12.5	3	0.249	24.9	0.000
4	25	3	0.249	24.9	0.533
5	50	3	0.249	24.9	0.867
6	100	3	0.249	24.9	1.000

AE 8/31/93

8505-093-124-032, 033

APPENDIX E
ANALYTICAL CHEMISTRY



Alden Analytical
Laboratories, Inc.

REPORT OF ANALYTICAL RESULTS

Client: ReTec	Alden Project Number: 9306016/1
Client Sample Number: LTF-1	Alden Sample Number: 4001
Date of Sample Receipt: 06/07/93	Analysis Method: EPA 8270
Date of Sample Extraction: 06/08/93	Matrix: Soil
Date of Sample Analysis: 06/15/93	Reporting Units: ug/kg

Compound Name	CAS No.	Reporting Limits (RL)	Reporting Results
Naphthalene	91-20-3	19000	29000
2-Methylnaphthalene	91-57-6	19000	33000
Acenaphthylene	208-96-8	38	1900
Acenaphthene	83-32-9	19000	89000
Dibenzofuran	132-64-9	19000	57000
Fluorene	86-73-7	19000	74000
Phenanthrene	85-01-8	19000	220000
Anthracene	120-12-7	19000	32000
Fluoranthene	206-44-0	19000	100000
Pyrene	129-00-0	19000	67000
Benzo(a)Anthracene	56-55-3	19000	16000*
Chrysene	218-01-9	19000	17000*
Benzo(b)Fluoranthene	205-99-2	77	7400
Benzo(k)Fluoranthene	207-08-9	77	5400
Benzo(a)Pyrene	50-32-8	77	5400
Indeno(1,2,3-cd)Pyrene	193-39-5	77	1500
Dibenz(a,h)Anthracene	53-70-3	77	510
Benzo(g,h,i)Perylene	191-24-2	77	1000

Surrogates	Amount Added	Percent Recovery	Recovery Limits
d5-Nitrobenzene	100 ug	65	23-120
2-Fluorobiphenyl	100 ug	100	30-115
d14-Terphenyl	100 ug	92	18-137

Total PAH = 559.7
Total CPAH = 54.2

* Value is below established reporting limits but is reported as an estimate due to a positive spectral match.

LTF-1 \Rightarrow composite of 5 sub-samples,
randomly selected, @ \approx 0-6"

AF 8/31/93


**ANALYTICAL
RESOURCES
INCORPORATED**

 Analytical
Chemists &
Consultants

**Final Report
Laboratory Analysis of Selected Parameters**
Matrix: SOIL

Project No: 9306016/1/3-0011

QC Report No: RBTBC - E053

Date Received: 06/09/93

Data Release Authorized: *MR. [Signature]*

Report Prepared 06/29/93 DWN

 333 Ninth Ave. North
Seattle, WA 98109-5187
(206) 621-8490
(206) 621-7523 (FAX)

Sample Data:		DATE OF ANALYSIS								
		6/18/93	6/11/93	06/11/93	06/11/93		06/22/93	06/16/93	06/23/93	06/12/93
		Method	EPA 160.3	EPA 350.1	EPA 354.1	EPA 353.2		EPA 351.4	EPA 413.1	EPA 365.2
Number		SM 2540 B	SM 4500-NH3 H	SM 4500-NO2 B	SM 4500-NO3 F	CALCULATED	SM 4500-Norg	SM 5520 B	SM 4500-P	SM 4500-P
Lab ID	Sample Number	TOTAL SOLIDS (%)	NH3-N (mg-N/kg)	NO2-N (mg-N/kg)	NO2+NO3-N (mg-N/kg)	NO3-N (mg-N/kg)	TKN (mg-N/kg)	FOG (mg/kg)	TOTAL-P (mg-P/kg)	ORTHO-P (mg-P/kg)
E053 A	40U1 A-C	86.46%	0.51	0.11	0.13	<0.11	184	2.290	685	0.38

Method Blank Analysis:

	TOTAL SOLIDS (%)	NH3-N (mg-N/l)	NO2-N (mg-N/l)	NO2+NO3-N (mg-N/l)	NO3-N (mg-N/l)	TKN (mg-N/l)	FOG (mg/L)	TOTAL-P (mg-P/l)	ORTHO-P (mg-P/l)
Method Blank 1	<1.0	0.045	<0.010	0.052	-	<0.1	1.1	<0.016	0.004
Detection Limit:	1.0	0.010	0.010	0.010	-	0.1	<1.0	0.016	0.004

Check Standard Analysis:

	(%)	(mg-N/l)	(mg-N/l)	(mg-N/l)	(mg-N/l)	(mg-N/l)	(mg/L)	(mg-P/l)	(mg-P/l)
Measured Value	-	10.1	9.70	8.96	-	10.3	58.5	0.080	0.042
"True" Value	-	10.0	10.0	10.0	-	10.0	62.3	0.080	0.040
% Recovery	-	101%	97.0%	89.6%	-	103%	93.9%	100%	105%

Duplicate Analysis:

	(%)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg/kg)	(mg-P/kg)	(mg-P/kg)
Sample ID	E053 A	E053 A	E053 A	E053 A	-	E053 A	E053 A	E053 A	-
Original	86.46%	0.51	0.11	0.13	-	184	2.290	685	-
Duplicate	86.33%	0.46	<0.11	0.11	-	189	1.850	681	-
RPD	0.15%	10.31%	-	16.67%	-	2.68%	21.3%	0.89%	-

Duplicate Analysis:

	(%)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg-N/kg)	(mg/kg)	(mg-P/kg)	(mg-P/kg)
Sample ID	-	E053 A	E053 A	E053 A	-	E053 A	E053 A	E053 A	-
Original	-	0.51	0.11	0.13	-	184	2.290	685	-
Spike	-	107	114	109	-	1,876	22,000	841	-
Spike Amount	-	116	116	116	-	2,230	20,600	60.9	-
% Recovery	-	91.8%	98.2%	93.9%	-	75.9%	95.7%	236%	-

Comments: Values are reported on dry weight basis.

Oil and Grease determined by partition gravimetric technique with soxhlet extraction.

Ammonia & Nitrate determined on 2M KCl extracts.

Ortho-P determined on 1:10 aqueous extract.

Total P spike masked by high sample background concentration.

*Final Nutrients for sample
Same composite*